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Amino Acid Substitution within the VP7 Protein of G2 Rotavirus Strains Associated with Failure To Serotype

MIREN ITURRIZA GÓMARA,[1]* DAVID CUBITT,[2] ULRICH DESSELBERGER,[1] AND JIM GRAY[1]

Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge CB2 2QW,[1] and Camelia Botnar Laboratories, Great Ormond St. Hospital for Children, London WC1N 3JH,[2] United Kingdom

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Rotavirus strains collected in the United Kingdom during the 1995-1996 season and genotyped as G2 by reverse transcription-PCR failed to serotype in enzyme-linked immunosorbent assays using three different G2-specific monoclonal antibodies. The deduced amino acid sequences of the antigenic regions A (amino acids 87 to 101), B (amino acids 142 to 152), and C (amino acids 208 to 221) of VP7 revealed that a substitution at position 96 (Asp→Asn) correlated with the change in ability to serotype these G2 strains.

Rotaviruses are triple-layered particles of the *Reoviridae* family that contain 11 segments of double-stranded RNA. The outer layer is composed of VP7 and VP4 proteins, encoded by gene segments 9, 7, or 8 (depending on the strain) and 4, respectively (8). These two proteins elicit neutralizing antibody responses and form the basis for the dual classification system of rotaviruses into G and P types, the designations being derived from glycoprotein (VP7) and protease-sensitive protein (VP4), respectively (8). Comparative sequence analyses of the deduced VP7 amino acid sequences of different animal and human rotavirus serotypes have identified six serotype-specific variable regions (VR) between amino acids (aa) 39 and 50, aa 87 and 101, aa 120 and 130, aa 143 and 152, aa 208 and 221, and aa 233 and 242, and these have been designated VR4 to VR9 (9, 11). VR5, VR7, and VR8 correspond to the antigenic regions A, B, and C, respectively, which have been confirmed as major rotavirus neutralization sites by mapping of neutralization escape mutants (6, 7, 16, 17).

Serotyping using G type-specific monoclonal antibodies (MAbs) has been applied widely in rotavirus epidemiological studies. However, the results of many studies have been incomplete due to the limited availability of MAbs specific for types other than G1 to G4, the relatively low sensitivity of the method due mainly to the requirement of intact virus particles, or to the existence of monotypes or antibody escape mutants within the different G types (2–4). Monotypes within G1, G2, G3, and G4 rotaviruses react with different degrees of affinity against different panels of G-specific MAbs (21) .

Previously we reported that rotavirus strains collected in the United Kingdom during the 1995-1996 season and genotyped by reverse transcription-PCR as G2 failed to serotype in enzyme-linked immunosorbent assays (ELISAs) using G2-specific MAbs (13). Complementary DNAs of the VP7 genes of a subset of these strains were partially sequenced and compared to corresponding sequences of a subset of successfully serotyped G2 strains collected during 1990 and 1991 in order to identify amino acid substitutions at the VP7 antigenic sites that may be responsible for the failure to react with different G2-specific MAbs.

G-serotyping ELISAs and genotyping reverse transcription-PCRs were performed as previously described (1, 10, 13) using 10% rotavirus-positive fecal suspensions in balanced salt solution. G-serotyping ELISAs (13) were performed using three different G2-specific MAbs (S2-2G10 [23], RV5:3 [5] and IC10 [20]). The presence of intact VP7 was confirmed in an ELISA using a cross-reactive MAb (MAb/60 [22]).

Twenty-one G2 rotavirus strains isolated in the United Kingdom—including (i) 10 rotavirus strains from the 1995-1996 rotavirus season which failed to serotype using all three G2-specific MAbs, (ii) 9 strains from an archival collection of rotaviruses from the season 1990-1991 that had been successfully serotyped using MAb RV5:3 (19), and (iii) 2 strains isolated during the 1998-1999 rotavirus season for which the serotype was not determined—were selected for sequencing of the VP7 cDNA.

Sequencing of the VP7 amplicons was performed using an automated sequencing system (Beckman CQ2000). Primers specific for conserved regions of the VP7 gene were used for amplification and sequencing of an 884-bp region of the gene: VP7-F (nucleotides 49 to 71), 5′ ATGTATGGTATTGAATA TACCAC 3′, and VP7-R (nucleotides 914 to 933), 5′ AACT TGCCACCATTTTTTCC 3′. Sequence data were analyzed using the SeqMan and Megalign (both of the DNAstar software package; Lasergene) software programs.

The presence of the VP7 protein in samples that failed to serotype was confirmed by reactivity in an ELISA using the cross-reactive MAb/60. None of the 10 G2 nonreactive specimens cross-reacted with G1-, G3-, or G4-specific MAbs (data not shown).

The alignment of the deduced amino acid sequences of the VP7 genes revealed amino acid substitutions at positions 87 (Ala→Thr) and 96 (Asp→Asn), both located within antigenic region A (aa 87 to 101). These were the only consistent differences between the strains that were successfully serotyped using MAb RV5:3 and those that failed to serotype with all three G2-specific MAbs (Table 1). Random mutations were found in antigenic sites B and C (Table 1) and in other areas of the sequenced VP7 fragment (data not shown) but were not significantly associated with the ability or inability to serotype.

* Corresponding author. Mailing address: Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Hills Rd., Cambridge CB2 2QW, United Kingdom. Phone: 44-1223-586925. Fax: 44-1223-242775. E-mail: mirren.iturriza-gomara@addenbrookes.nhs.uk.

TABLE 1. Deduced amino acid sequences of the VP7 antigenic site A of rotavirus G2 strains[a]

Strain	Antigenic site A (aa 87–101)															Antigenic site (aa 142–152)										Antigenic site C (aa 208–221)													
DS-1	A	E	A	K	N	E	I	S	D	D	E	W	E	N	T	M	R	Y	D	N	T	S	E	L	D	A	K	T	T	D	V	N	T	F	E	I	V	A	S	S
S2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.
RV5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1076-Aus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	S	.	.	T	.	.	.	.	.	.
Hu/Aus/5/77	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
92A-Aus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.
HN 126	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC3/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC2/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC4/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC12[b]/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC14/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC15/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC17[b]/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DC24/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.
DC22/91	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
543/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.
544/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
563/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
583/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
538/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
621[b]/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
625/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
366/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
617/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
351/96	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
399/99	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
721/99	T	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

[a] Prototype strains and sequences obtained from GenBank/EMBL are in roman type, and strains found in the United Kingdom that were serotyped are in boldface type. Accession numbers are as indicated: S2, VGXRS2; DS-1, VGXRD; HN 126, VGXRHN; Hu/Aus/5/77, X00572; 92A-Aus, U73947. RV-5 and 1076 were obtained from Coulson et al. (4). A dot indicates homology with the DS-1 sequence at that position.

[b] Sequence of antigenic site C not available.

The loss of reactivity with G2-specific MAbs (RV3:1 and RV5:4) of one G2 rotavirus strain isolated in Australia (strain 1076) had previously been correlated with amino acid substitutions in antigenic regions B and C, at positions 147, 213, and 217 (4). However, in our study no consistent differences were found in antigenic regions B and C between strains that were serotyped and those that failed to serotype (Table 1). The sequences of antigenic region A of the strains which were successfully serotyped were identical to those of the prototype G2 strains RV5, S2, and DS-1 and two human strains which had been serotyped successfully in Australia (Human/Australia/5/77 [Hu/Aus/5/77] and 92a-Australia [92A-Aus]) (Table 1). The prototype serotype G2 strain HN126 (11) typeable by G2-specific MAbs possessed a single amino acid substitution, Ala→Thr at position 87 (Table 1), strongly suggesting that this amino acid substitution is therefore not responsible for the antigenic change leading to a failure to react with G2-specific MAbs.

Thus, the amino acid substitution at position 96 (Asp→Asn) is likely to be responsible for the failure to react with the G2-specific MAbs. All strains sequenced with an amino acid substitution at position 96 also had an amino acid substitution at position 87. The substitution at position 96 in the absence of a substitution at position 87 has been found in experiments with rotavirus antibody escape mutants (MAb RV5:4) (17). This substitution induces a change in electric charge and may result in a conformational change of the epitopes recognized by neutralizing antibodies. Although the epitopes recognized by rotavirus neutralizing antibodies have not been completely defined, it has been proposed that antigenic regions A and C which are distant in the linear molecule interact closely together in the folded form of the VP7 molecule (7).

The sequences of VP7 antigenic region A of the strains isolated during 1998 and 1999 were identical to those isolated in 1995 and 1996, and it appears that these two substitutions are being maintained over time. These mutants can therefore be regarded as antibody escape mutants that are widely dispersed geographically and are identical to G2 strains isolated post-1993 in Taiwan, where an epidemic caused by G2 rotavirus strains in 1993 was reported (24). It was suggested that the epidemic in Taiwan was associated with an alteration in pathogenicity, perhaps conferred by reassortment (24). However, the explosive reemergence of G2 strains in this region may have been due to immune evasion as a consequence of altered antigenicity conferred by the amino acid substitution at position 96 of antigenic region A. The lack of cross-protection conferred from previous infections with G2 strains may also explain the higher incidence of infection with rotavirus G2 strains found in the older population in the United Kingdom between 1995 and 1999 (12).

Genetic drift through the accumulation of point mutations (14) and genetic shift through reassortment (15) are thought to be the major mechanisms associated with rotavirus evolution. Although some point mutations appear to be localized both

temporally and geographically (14), others become stable, giving origin to global genetic lineages (15, 18) and monotypes of the different G and P types circulating synchronously worldwide. In this context, the usefulness of the G-type-specific MAbs is likely to be time limited as a result of the accumulation of point mutations at their corresponding epitopes.

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REFERENCES

1. **Boom, R., C. J. A. Sol, M. M. M. Salismans, C. L. P. M. E. Jansen, P. M. Wertheim-van Dillen, and J. van den Noordaa.** 1990. Rapid and simple method for the purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
2. **Coulson, B. S.** 1996. VP4 and VP7 typing using monoclonal antibodies. Arch. Virol. Suppl. **12:**113–118.
3. **Coulson, B. S., and C. Kirkwood.** 1991. Relation of VP7 amino acid sequence to monoclonal antibody neutralization of rotavirus and rotavirus monotype. J. Virol. **65:**5968–5974.
4. **Coulson, B. S., C. D. Kirkwood, P. J. Masendycz, R. F. Bishop, and G. Gerna.** 1996. Amino acids involved in distinguishing between monotypes of rotavirus G serotypes 2 and 4. J. Gen. Virol. **77:**239–245.
5. **Coulson, B. S., L. Unicomb, G. Pitson, and R. Bishop.** 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. J. Clin. Microbiol. **25:**509–515.
6. **Dyall-Smith, M. L., and I. H. Holmes.** 1984. Sequence homology between human and animal rotavirus serotype-specific glycoproteins. Nucleic Acids Res. **12:**3973–3982.
7. **Dyall-Smith, M. L., I. Lazdins, G. W. Tregear, and I. H. Holmes.** 1986. Location of the major antigenic sites involved in rotavirus serotype-specific neutralization. Proc. Natl. Acad. Sci. USA **83:**3465–3468.
8. **Estes, M.** 1996. Rotaviruses and their replication, p. 1625–1655. *In* B. N. Fields et al. (ed.), Fields virology, 3rd ed. Lippincott-Raven, Philadelphia, Pa.
9. **Glass, R. I., J. Keith, O. Nakagomi, T. Nakagomi, J. Askaa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1985. Nucleotide sequence of the structural glycoprotein VP7 gene of Nebraska calf diarrhea virus rotavirus: comparison with homologous genes from four strains of human and animal rotaviruses. Virology **141:**292–298.
10. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z. Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28:**276–282.
11. **Green, K., K. Midhun, M. Gorziglia, Y. Hoshino, A. Kapikian, R. Chanock, and J. Flores.** 1987. Comparison of the amino acid sequences of the major neutralisation protein of four human rotavirus serotypes. Virology **161:**153–159.
12. **Iturriza-Gómara, M., J. Green, D. Brown, M. Ramsay, U. Desselberger, and J. Gray.** 2000. Molecular epidemiology of human group A rotavirus infections in the United Kingdom between 1995 and 1998. J. Clin. Microbiol. **38:**4094–4401.
13. **Iturriza-Gómara, M., J. Green, D. W. Brown, U. Desselberger, and J. J. Gray.** 1999. Comparison of specific and random priming in the reverse transcriptase polymerase chain reaction for genotyping group A rotaviruses. J. Virol. Methods **78:**93–103.
14. **Iturriza-Gómara, M., J. Green, D. W. Brown, U. Desselberger, and J. J. Gray.** 2000. Diversity within the VP4 gene of rotavirus P[8] strains: implications for reverse transcription-PCR genotyping. J. Clin. Microbiol. **38:**898–901.
15. **Iturriza-Gómara, M., B. Isherwood, U. Desselberger, and J. Gray.** 2001. Reassortment in vivo: a driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. J. Virol. **75:**3696–3705.
16. **Kirkwood, C., P. J. Masendycz, and B. S. Coulson.** 1993. Characteristics and location of cross-reactive and serotype-specific neutralization sites on VP7 of human G type 9 rotaviruses. Virology **196:**79–88.
17. **Lazdins, I., B. S. Coulson, C. Kirkwood, M. Dyall Smith, P. J. Masendycz, S. Sonza, and I. H. Holmes.** 1995. Rotavirus antigenicity is affected by the genetic context and glycosylation of VP7. Virology **209:**80–89.
18. **Maunula, L., and C. H. von Bonsdorff.** 1998. Short sequences define genetic lineages: phylogenetic analysis of group A rotaviruses based on partial sequences of genome segments 4 and 9. J. Gen. Virol. **79:**321–332.
19. **Noel, J. S., S. P. Parker, K. Choules, A. D. Phillips, J. Walker Smith, and W. D. Cubitt.** 1994. Impact of rotavirus infection on a paediatric hospital in the east end of London. J. Clin. Pathol. **47:**67–70.
20. **Padilla-Noriega, L., C. F. Arias, S. López, F. Puerto, D. R. Snodgrass, K. Taniguchi, and H. B. Greenberg.** 1990. Diversity of rotavirus serotypes in Mexican infants with gastroenteritis. J. Clin. Microbiol. **28:**1114–1119.
21. **Raj, P., D. O. Matson, B. S. Coulson, R. F. Bishop, K. Taniguchi, S. Urasawa, H. B. Greenberg, and M. K. Estes.** 1992. Comparisons of rotavirus VP7-typing monoclonal antibodies by competition binding assay. J. Clin. Microbiol. **30:**704–711.
22. **Shaw, R., D. Stoner-Ma, M. Estes, and H. Greenberg.** 1985. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. J. Clin. Microbiol. **22:**286–291.
23. **Taniguchi, K., T. Urasawa, Y. Morita, H. Greenberg, and S. Urasawa.** 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. **155:**1159–1166.
24. **Zao, C. L., W. N. Yu, C. L. Kao, K. Taniguchi, C. Y. Lee, and C. N. Lee.** 1999. Sequence analysis of VP1 and VP7 genes suggests occurrence of a reassortant of G2 rotavirus responsible for an epidemic of gastroenteritis. J. Gen. Virol. **80:**1407–1415.